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THE SIGNIFICANCE OF BACILLUS COLI IN DRINKING WATER.

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OF late years the bacteriological examination of water has become of greater importance in routine work and more widely employed. With this development it has become increasingly apparent that the mere number of organisms present in a given quantity of water, though of considerable value, is liable to cause many fallacies if solely relied upon.

The *kinds* of organisms present are of greater significance. It is impossible to work out and determine all the organisms present, nor is it necessary, so the organisms especially associated with sewage and harmful contamination in general, become of especial importance. Of such organisms the *Bacillus coli* is considered by most workers to be among the most valuable. In itself as met with in water it is probably quite harmless, but as an indicator of contamination its value has been rated very highly.

As to the exact significance to be ascribed to this organism authorities however differ widely, and the opinions held range on the one hand from those who consider its presence as significant of contamination without regard to the number of such *B. coli* present, to those who reject its detection as of no value because of its wide distribution in nature and the possibility of its being derived from apparently harmless sources.

That such a wide discrepancy of opinion exists is seen when the following results and opinions held by different workers are considered.

E. O. Jordan ⁽¹⁾ objects to *B. coli* as a true index of sewage contamination because he has found "in spring water which was beyond any

suspicion of contamination bacteria which in form, size, growth on gelatine, potato, etc. were indistinguishable from *Bacillus coli communis*."

Theobald Smith⁽²⁾ says "it is safe to infer that an organism which is so uniformly present in the intestinal tract, and which possesses to a slight degree pathogenic powers, really belongs there, and that its presence outside of the intestines in soil and water may be regarded as due to the continual contamination with faecal discharges of men and animals. Either through the presence of sewage in the water or through the washing into streams of surface soil from manured ground the colon bacilli enter streams and thus become a valuable index of that kind of pollution which we should most carefully guard against."

Weissenfeld⁽³⁾ found a coli-like organism in good and bad waters, using 1 litre as the standard amount to examine. His cultural and other characters descriptive of *B. coli* are however by no means conclusive.

Other observers lay stress upon the quantitative enumeration of the bacillus. Thus Horrocks⁽⁴⁾ states, "I would say that a water which contained *B. coli* so sparsely that 200 c.c. required to be tested in order to find it, has probably been polluted with sewage, but the contamination was not of recent date."

Pakes⁽⁵⁾ asserts that, "Drinking water from a deep well should contain no *B. coli* in any quantity: water from other sources which contains the *B. coli* in 20 c.c. or less should be condemned; that which contains the organism in any quantity between 20 and 50 should be looked upon as suspicious, between 50 and 100 as slightly suspicious, and only in greater quantity than 100 c.c. as probably safe."

Houston⁽⁶⁾ has quite recently very clearly stated this quantitative view of the matter, pointing out the value of the presence of *B. coli* if the relative numbers present are considered.

Other authors, viz. Chick and Boyce^{(7) (8)}, have contented themselves with examining small quantities only of water, and base their views on the presence or absence of this organism in 1 c.c. of the water. With such divergent views there is obviously room for careful, renewed investigation.

In such an investigation the three following points must, in my opinion, always be kept in view:—

(1) That it is not so much the presence of *B. coli* which is of possible value but the *number* present.

The consideration of the problem is essentially quantitative, and the

number of *B. coli* in a standard amount of water must be considered. This view is insisted upon by many English bacteriologists and there can be no doubt of its vital importance in studying the question.

(2) That the kind of water in which this organism is found is of great importance, and that a number of *B. coli* per litre may be passed as safe, or at least as no evidence of dangerous contamination, in a water from one source which would be sufficient to condemn the water from another source.

(3) The exact point on the water supply from which the sample is collected is of great importance. Frequently results differing considerably are obtained with samples taken from source and tap respectively, even with quite unfiltered waters; and with arbitrary standards one would be condemned and the other passed as satisfactory. Illustrations of this are furnished in the following tables.

This factor though very generally known is frequently ignored by many of those writing on this question.

To study the subject to the best advantage it is necessary to have full and reliable information as to the source and nature of the water of which samples are examined.

Chemical analyses of an exactly similar sample collected from the same place and at the same time are also of great value for comparison. In other words, all three methods of examination should be employed, *i.e.* the sanitary, the bacteriological, and the chemical.

For all the waters referred to in this paper very great care was taken to obtain satisfactory information as to the exact nature of the water supply, its possibilities of contamination, and any particulars likely to be of use in the investigation. A considerable number of samples are not included because full particulars were not available.

I personally inspected and investigated several of the most important water supplies, taking my own samples.

The information for the others was derived from reliable sources, and I wish here to record my thanks to Dr W. Williams, Medical Officer of Health of the Administrative County of Glamorgan, for the trouble he has taken in supplying me with much information in regard to the water supplies examined, and for the care which he has taken to verify any doubtful points, such as exact source, or possibilities of contamination. His unrivalled knowledge of the water supplies of Glamorgan has been of great value in obtaining accurate information. The chemical analyses were all performed in the Cardiff and County Public Health Laboratory, some of them by myself but the majority by Mr. Sugden,

B.Sc., F.I.C. Assistant Bacteriologist, to whom I am indebted for much careful and accurate work.

To arrive at the significance of *B. coli* in water I have studied a large number of public supplies, at different times and taken from different parts of the water system. Local wells and other small supplies have also been investigated.

The method used to examine the samples for *B. coli* only requires a brief notice here as it is fully described elsewhere⁽⁹⁾. It consists in incubating varying quantities of the water at 37° C. with ordinary broth containing $\frac{1}{2}$ per cent. glucose and 1.2 per cent. of a $\frac{1}{2}$ per cent. watery solution of neutral-red.

If *B. coli* is present the red changes to yellow or orange and a marked fluorescence develops. To isolate the organism and to make certain that it was present, in all but a small proportion of cases the yellow fluid was brushed over agar or gelatine plates and the *B. coli* isolated and its characters worked out.

In all cases except those otherwise indicated this is the course that has been taken. If no reaction developed in any of the tubes the largest amount of water was in the same way brushed and examined for *B. coli*, in all cases with a negative result.

As a rule, but not quite invariably, the largest amount of water (*i.e.* the 40 c.c.) was brushed when a complete negative result was obtained, and the smallest amount giving positive result (*i.e.* change to yellow and fluorescence) for the waters giving a positive reaction.

It should also be stated that for the bacteriological examinations very great care was taken to have media of standard reaction.

Both the agar and gelatine were standardised to a +1.0 per cent. reaction (as recommended by Dr Eyre)⁽¹⁰⁾.

The agar plates were counted after about 40—46 hours' incubation and the gelatine daily for as long as possible (*i.e.* until liquefied). This usually represented a 3 to 4 days' count.

The different supplies examined, with data as to their nature and possibilities of contamination, and results of chemical and bacteriological examinations, will be considered first. Pure upland surface waters will be first described.

Supply No. 1. This is the most important public supply in Glamorganshire. The water is a typical upland surface water, the gathering grounds being in Brecknockshire, quite near the Brecknock Beacons and over 30 miles from the town supplied.

The collecting areas are two in number. The larger (Reservoir A) has a water

collecting area of 51 acres; and the reservoir, which is at an elevation of 1340 ft., has a capacity of 345 million gallons. The area of the other collecting area is 45 acres; and the reservoir (Reservoir B), which has an elevation of 1073 ft., has a capacity of 322 million gallons.

The water is carried from these (excluding the several balancing reservoirs *en route*) to two large storage reservoirs a few miles from the main town supplied. The distance from the lower collecting reservoir (Reservoir B) to the storage reservoir is 32 miles. The storage reservoirs have a capacity of 317 million gallons (Reservoir C) and 80 million gallons (Reservoir D) respectively. From these the water passes to the filter-beds (at E and F), where it is filtered through sand and then distributed.

The E. filter-beds are 6 in number, each capable of filtering 1 million gallons per day. At F they are 5 in number. The water is screened a large number of times before the final filtration. The filtration cannot be considered very efficient. When the sand becomes dirty and the filtration slower the top layers are removed and washed. In other words the filtration is mainly mechanical and only to a small extent *vital*.

With regard to possibilities of contamination, the gathering areas are among the Brecknock hills and quite remote from all habitations. The nearest town is 6 miles away, and within that distance there are only a few scattered farms. There is only one house on each gathering area. For the lower one this is the house of the waterworks keeper; earth-closets are used and the most rigid precautions are taken. Contamination from this source is impossible. On the upper reservoir (Reservoir A) there is only one house, and the drainage of this certainly finds its way into the reservoir. This is the only possible source of contamination to either reservoir from human sources. It is a very small one, and the results of the examinations made show that it is quite unimportant as regards the points at issue.

The gathering areas are ordinary hill side, and there are *no* manured fields or cultivated land on them. The entering streams rise in the hills round and can be traced to their sources. The gathering areas are undoubtedly among the best in the country, with no possibility of human contamination except the small source indicated.

Sheep however are on the gathering area all the year round. The two lower storage reservoirs (C and D) only contain the water from these two collecting reservoirs; *i.e.* all the streams around them are very carefully led away and prevented from entering these reservoirs.

Wild duck and other birds can frequently be seen in large numbers on the water. There is a probability of contamination therefore from animal sources.

I have personally inspected all the reservoirs, gathering areas and filter-beds and satisfied myself as to the correctness of the above description.

The significance of *B. coli* in such a water is therefore a fairly straightforward one, and a large number of samples were examined. The results obtained are given in Tables I, II, and III. In Table IV a selection of the results of chemical analyses of this water is given for comparison.

Supply No. 2. Also a typical upland surface water and an important public supply, supplying a population of about 100,000 people. The gathering area is in Glamorganshire, at a considerable elevation. The main reservoir is formed by a dam on a small river, which rises some distance further away among the hills,

quite away from possibilities of human contamination. The gathering area has only one house on it, which is $\frac{1}{2}$ a mile *below* the upper reservoir, is used by the two men connected with the waterworks, and from which contamination is rigidly excluded. The surrounding hills have only a few tracks and no roads, and are only used for sheep. There are no manured fields or cultivated land. By an overflow weir a large quantity of water passes out from the main reservoir, and makes the river. This river is again partially blocked about $1-1\frac{1}{2}$ miles lower down to form another reservoir. The water from this lower reservoir is filtered through sand in beds quite near by and is then distributed. The water from the upper reservoir passes by pipes to near the sand filter-beds, where it is filtered through quartz-filters, the water passing into a covered reservoir.

This gathering area was personally inspected by Dr Williams and myself in Dec. 1901. The only possible source of contamination was from the excreta of animals or it may be from the soil. Sheep were allowed and could be plentifully seen all over the gathering area; and considerable quantities of sheep dung were seen, some quite close to the water and washed by it. A number of samples of water were collected (Nos. 8—14, incl. Table V), No. 8 from the upper reservoir, No. 11 from the river midway between the two reservoirs, Nos. 10, 12, 13 from different little mountain streams the sources of which could be readily traced some distance away among the uplands. All three entered the river between the reservoirs. No. 14 was collected *in* the quartz-filters just before filtration, and No. 9 from a little patch of marsh water (there had been much rain a few days previously) about 200 yards below the upper reservoir and a few yards from the river.

A sample of soil was also collected from just by the side of No. 12 stream. The soil was moist and peaty and contained much vegetable matter.

The results obtained from these and other samples are given in Tables V and VI.

Supply No. 3. This is a proposed water supply. The samples were collected by Dr W. Williams, to whom I am indebted for the following description.

The proposed collecting area is 2300 acres, and consists of a natural basin through which the river runs. The area is entirely on old red sandstone and there is no peat. The rock appears on the surface everywhere and along the bed of the river. The basin is bounded by hills, under the crest of which appear numerous springs. The sides of the basin are markedly furrowed by springs, the water of which flows into the river. The river rises at the head of the basin in a number of small streams at an elevation of 1500 ft. The area shows evidence of cow dung and plenty of sheep dung. Sheep and other animals are allowed on all the summer. Samples collected Jan. 13th, 1902.

There are no houses on the area, and Dr Williams informs me that apart from animal excreta there is no possibility of any contamination and that it is one of the very best gathering areas with which he is acquainted. Six samples were collected: No. 1 and No. 2 from the river on each side of the gauge. No. 4 from the river at the top of the proposed reservoir, *i.e.* about $\frac{3}{4}$ mile above the gauge. No. 3 was from a streamlet having its origin in a spring about 400 yards away. As collected, the sample consisted mainly of spring water but with some upland surface water. No. 5 and No. 6 were pure spring waters collected from the upper part of the gathering area from 2 springs. These samples were collected as the water came out from the ground. The results obtained are given in Table VII.

No chemical examinations were made.

A number of other entirely upland surface waters were also examined but not in such detail, so that a shorter description will answer every purpose. These waters include Nos. 4 to 9 inclusive; and the results of the bacteriological and chemical analyses are given in Tables VI, VIII, IX and X.

Supply No. 4. Not a large supply. Supplies a population of about 8000. A very limited gathering area from which the upland surface water collects into a reservoir. This is a few miles from the town. One small house only on the gathering area, but no cultivated land. Soil very peaty, and sheep and other animals graze on the gathering area. The water is not filtered.

Supply No. 5. An upland surface water collected from peaty mountain land. There is one farm-house and a few manured fields on the area, and numerous sheep graze over it. The area is surrounded by trees and by vegetation, and cannot be considered a good gathering area. There are also a number of old colliery workings which may contain excrement. Water is sand-filtered.

Supply No. 6. An upland surface water entirely, all the water being derived from the surface of hills. The whole of the gathering ground is entirely devoid of dwelling-houses. A few mountain paths traverse the collecting area, and are not infrequently used by colliers and others. The uplands serve as grazing ground for hundreds of sheep, which are the only quadrupeds to be found on the whole area. Before distribution the water is filtered through sand. The subsoil of the collecting area is carboniferous limestone, and distributed over the area are a few very small "pockets" of peat.

Supply No. 7. An upland surface water with collecting area of about 480 acres. Water liable to pollution from manured and ploughed fields or a few farms on the area. Water has often a distinct yellow tint, and the storage reservoir has a thick layer of peat at the bottom. Filtered through sand before distribution.

Supply No. 8. An upland surface water which is collected into an open reservoir and then distributed. Sand-filtered before distribution. A certain amount of spring water also is collected into the reservoir.

Supply No. 9. A large upland surface water which supplies a population of over 100,000 people. It is of interest in being an upland surface water undoubtedly exposed to contamination. There are three collecting reservoirs, of which the two upper, A or B, are several miles above the lower, and are not liable to any pollution other than from sheep. The third reservoir (Reservoir C) is on the same river but lower down, and is liable to distinct contamination from four separate farms. From all the four farms the contamination is distinct and gross in nature. Thus for one, a stream feeding the main river passes through the farm-yard, where there are accumulations of manure. For two others there are accumulations of manure within a few yards of streams feeding the main river; while for the fourth, not only does manure drain into a feeding stream but also the contents of the slop water drains. The river receives all but one of these streams before it is again blocked to form Reservoir C; which reservoir also receives direct the fourth contaminated stream.

The water from the two upper reservoirs (A and B) is filtered about six miles from the source and supplied to one part of the town. The water from Reservoir C is also filtered about six miles away and supplies the rest of the town. I am

indebted to the Medical Officer of Health of this town for full particulars and plans of the different streams and reservoirs.

A number of water supplies were also examined which are neither pure upland surface water nor entirely spring water, but which are mainly springs, but supplemented by upland waters. Particulars of a few of these are given briefly. The results of the bacteriological and chemical analyses are given in Tables XI and XII.

Supply No. 10. Partly a spring, partly an upland surface water from the hill sides. Collected direct into a reservoir but not in any way filtered. The samples were taken from a tap about two miles from the source. Sheep over the hills. A small supply.

Supply No. 11. Another small supply, partly upland surface and partly from some small springs. The water is filtered through about 3 ft. of gravel and sand, and is then stored in a closed tank before distribution. The gathering area is partly peaty and is used for sheep, but is quite free from manured or cultivated fields.

Supply No. 12. This water supply consists of spring and upland surface water. The water from the several springs runs in a channel or brook for about a mile before it enters the service tank from which it is distributed. In its passage along the channel the water is supplemented by streams from the surrounding hills. On these hills there are a few sheep and sometimes a few horses and cattle but no cultivated fields. No possibility of sewage or human contamination. The water is not filtered in any way.

Supply No. 13. A quite small supply. Upland surface and spring. Sheep the only possible contamination. Unfiltered.

Supply No. 14. Another small supply. Mainly springs on mountain side. A small mountain stream is also taken in. Filtered through sand-filter. Sheep on the uplands as usual, and this the only possible source of contamination. No houses or cultivated land.

Pure Spring or Deep Well Waters.

Not a large number were examined and worked out, if a large supply of doubtful origin (No. 20) is excluded. The results obtained are given in Tables XIII and XIV.

Supply No. 15. This water supply is obtained from two springs in pennant sandstone, the water being impounded into a properly constructed reservoir. The reservoir is a covered one and no surface water can obtain admission into it. A pure spring water. It is distributed unfiltered.

Supply No. 16. This small supply is obtained from a spring, and no surface water gains access to it. The water is conveyed from the source by cast-iron pipes to a storage tank. It is not filtered in any way and there are no possibilities of contamination from human or animal sources.

Supply No. 17. Under this head are included a number of isolated supplies. Each was only examined once, *i.e.* each represents a quite separate supply. A considerably larger number of springs were examined, but only those in which full particulars were obtained and in which the examination for *B. coli* was thoroughly worked out are included.

Shallow Wells.

Only those are mentioned for which particulars are available. Under supply No. 18 are grouped a number of separate supplies. The numbers correspond to the numbers in Table XV.

Supply No. 18. (1) A shallow well said to be about 30 ft. deep. It is situated close to a chapel burial-ground and therefore regarded with suspicion. The strata consist of a loose sandstone gravel with a few beds of clay. I am informed that the water has been analysed two or three times during the last three years and the report has always been favourable.

(2) and (14) A surface well about 30 ft. deep. Surface water said not to gain access. Has a pump. Possibilities of contamination present.

(3) A shallow well in a peaty soil and close to the road. Known to be liable to contamination.

(4) A well in a clay soil. Probably polluted from slop water.

(5) A surface well, said to be liable to organic and vegetable pollution.

(6) A well, probably a shallow one. Walls not properly built and surface water not kept out. Nearest house 60 yards.

(7) A shallow well. Collected from pump.

(8) A shallow well situated upon a common. No houses near, and no liability to contamination. Properly covered in and a pump fixed.

(9) An open and shallow well.

(10) An old well about 15 ft. deep in a gravel soil. A row of eight houses near the well and a slop drain passes within three yards of it. Old privy pits 50 yards away. Covered over but surface water gets in.

(11) A well water. Doubtful if a shallow or deep well, but surface washings can and do gain access.

(12) A surface well in a clay and peat soil. Water examined because three Enteric Fever cases among those drinking the water.

(13) A well used by two persons suffering from Enteric Fever. The well is in the middle of a farm-yard. The sides and roof are defective and pollution can take place.

Supply No. 19. An important shallow well supply. The water is conveyed direct from the well in 2-inch pipes to a pump by the Town Hall, a distance of about 250 yards. Surface water is received only when the spring is disturbed by floods. No known sources, I am informed, of contamination, human or animal.

Supply No. 20. This supply will be considered by itself, as it is of a somewhat peculiar nature. It is a large supply and serves about 30,000 people. An underground supply tapped by a well 32 ft. deep and by a long (200 yards) lateral heading from this. The heading runs 25—30 ft. under the surface. The average quantity supplied per day is about 600,000 gallons. The water is pumped from the well into three reservoirs and is then distributed. It is not filtered in any way.

This supply cannot be said to have a gathering area, but the ground over the heading and round the well and pumping station is liable to flooding from a brook which runs in a semicircular fashion round it, and distant about 1200 ft. from the pumping station. This brook is contaminated by houses higher up and its chemical and bacteriological analysis shows marked evidence of pollution.

This area is usually moist, shows marked vegetable growth (grass, etc.), and is in winter frequently flooded and under water for days together. Also cattle and other animals graze over this area during part of the year (it is now enclosed and no animals allowed since October 1901). The exact classification of this water is not easy to decide. It is said to be a deep well water, though it is more correct apparently to call it an underground river. The well is sunk for 32 ft. and the soil from above down is surface soil, blue clay, peat, blue clay, red marl with stone and clay, hard limestone with banks of yellow marl.

The significance of *B. coli* in upland surface waters will first be considered. For this purpose supplies Nos. 1 to 9 are available. It will be noticed that this organism is present in all of them and in the great majority of the samples examined. It is further often present in considerable numbers. The source of these *B. coli* is more readily studied by considering the examinations made at the sources of the different supplies.

In supply No. 1 it will be seen that out of 17 examinations at the source (Table I) this organism was found and isolated in all but 3 (or 4 if a doubtful *B. coli* is excluded) when 40 c.c. was the amount examined: in 9 samples it was found in 10 c.c.; and in 2 c.c. in nearly half the samples in which that amount of water was examined. It was also found in 1 c.c. but never in $\frac{1}{2}$ c.c. It was present in both reservoirs and in all the entering streams examined.

In supply No. 2, six samples were in connection with the source (i.e. Nos. 8, 10, 11, 12, 13, 14), and *B. coli* present and isolated from five. The small entering upland streams are of particular interest as they could be traced to their respective sources. In one of them *B. coli* was present in 2, 10 and 40 c.c., in another in 40 c.c. only, while in the third it was absent in the 50 c.c. examined.

Supply No. 3 gives the results of examinations made of a proposed supply, one of exceptional purity or freedom from contamination. Even in this water *B. coli* was isolated in 4 out of the 6 samples, and in one was present in as little as 2 c.c. It is noteworthy that the two waters in which it was absent were both pure springs free from admixture with upland surface water.

The results of the examination of the sources of other upland surface supplies gave very similar results; and the investigation of samples other than at the source shows also how comparatively numerous this organism may be in this class of waters.

Supply No. 9, a large and important supply, gives a still larger number of *B. coli* present in the different samples. This supply however shows on inspection distinct possibilities of contamination

from farms in the neighbourhood of the reservoirs, and in the gathering area.

B. coli, then, appears to be habitually present in upland surface waters if a sufficient quantity of the water is examined, and further it is not infrequently present in considerable numbers and that in waters which appear to be absolutely free from sewage or human pollution and which on chemical analysis show no evidence of contamination.

What is the source of these *B. coli*? Bacilli so constantly present cannot be fortuitous. They are not natural constituents of water. Their presence can only be due to contamination of the water from some source in which they are numerous.

The possible sources of such contamination can only be the following:—

- (1) From human or sewage pollution.
- (2) From the washings of cultivated soil and manured fields.
- (3) From the washings of ordinary uncultivated upland soil.
- (4) From the excreta of animals grazing on the gathering grounds.

Regarding the first three supplies as being more completely investigated than the other sources, (1) and (2) can be quite excluded considering the nature of the gathering areas, while many of the small streams were traced to their source and shown to be quite free from the possibilities of such contamination. With regard to the possibility of *B. coli* being derived from uncultivated upland soil Houston (11) in his extensive investigations on this bacillus in soil says his results "seem to show conclusively that *B. coli* (or its close allies) is not discoverable or is present in small numbers only in *pure* soils. Further they would seem to indicate that *B. coli* is not readily isolated even from soils polluted with objectionable animal matters unless indeed the contamination is gross in amount and of *recent* sort." In another report the same author states that soil recently polluted with faecal matter will yield *B. coli* in washings therefrom, but other sorts will not yield *B. coli* in any large numbers.

In 15 samples of moorland soil H. Chick (7) found *B. coli* absent in all in the amounts examined, *i.e.* 0.1 to 0.02 gm. In the soil from supply No. 2 (personally collected, *vide supra*) a typical *B. coli* was isolated from 1 loop of the soil. The sample was taken after the upper layer had been rejected, but was quite close to a mountain stream (sample No. 12) and was very moist.

Examination of a small number of pure and contaminated soils for this organism has given me results somewhat similar to those of

Dr Houston, and it may probably be taken as fairly assured that if present in ordinary hillside soils this organism has been derived from animal excreta.

The source of the *B. coli* in these waters can with considerable certainty be ascribed to contamination of the water with animal excreta either directly or indirectly through the intermediary of the soil. Sheep are allowed to graze on all the gathering areas with which I am acquainted, and in all waters from such areas *B. coli* are present, often in considerable numbers. The excreta of sheep can usually be seen all about such water supplies and frequently washed by the water. Such excreta teem¹ with *B. coli* indistinguishable as far as I am aware from *B. coli* obtained from other sources. Five *B. coli* isolated at different times from such sheep dung showed characters quite like those of the ordinary *B. coli* found in water.

Sample No. 9, supply No. 2 (Table V), is of especial interest. The sample was personally collected from a patch of marshy water a few yards from the river. *B. coli* was present in 2, 10 and 40 c.c. Unfortunately smaller quantities were not examined. The considerable number of *B. coli* in this marsh water gives some clue as to the probable method of multiplication of this organism and how it gains access to the water.

Sheep dung is deposited in the stagnant water and such a water rich in organic matter soon swarms with *B. coli*. The next rain washes these into the nearest streamlet and so into the reservoir. The experimental possibility of this occurrence was demonstrated in the laboratory. A 5 litre glass jar was plugged with cotton-wool and sterilized, 4 litres of tapwater were added and the whole again sterilized. About 1½ grm. of sheep dung was added and the jar kept in the outside air and the temperature taken daily. Weather very cold with nightly frosts and the maximum temperature of the experiment about 9° C. Examined after a week and after a fortnight, and very numerous *B. coli* found in the water.

After these organisms gain access to a water supply they probably do not multiply but tend to gradually die out, presumably from the influence of unsuitable environment, the action of gravity, and the competition of the ordinary water micro-organisms.

That this is probable is shown to a certain extent by the results of the analyses. Thus for supply No. 1 the number of *B. coli* as well as

¹ Only one enumeration of the number of *B. coli* in sheep dung was made. In this case it was found that 1 grm. of the fresh dung contained about 280,000 *B. coli*.

of other organisms is generally less in the lower storage reservoirs than in the collecting reservoirs. Also if this organism was capable of considerable multiplication in stored water it is to be expected that the number of *B. coli* in the reservoirs would be much greater than in the main entering streams; but this is not so, the results of the analyses made of such streams showing sometimes more *B. coli* and sometimes fewer. Further, the result of an experiment made with Reservoir C negatives the probability of multiplication. On March 1st, 1902, I collected three samples from Reservoir C, taken respectively, No. 1 from the inlet, No. 2 from the middle (1 foot below the surface), and No. 3 from the outlet. The result of the examinations gave the following figures.

	No. 1	No. 2	No. 3
Developing at 37° C.	28	2	7
" " 20° C.	590	128	121
Number of <i>B. coli</i>	In 2, 10 & 40 c.c.	In 40 c.c. not in 2 or 10 c.c.	Not in 2, 10 or 40 c.c.

The few laboratory experiments made (six in all) confirm this as far as artificial conditions are available for comparison. Further experiments are being carried out. They show that *B. coli* (cultures recently isolated from water and from sheep dung were used) kept in sterile water in Winchester quart bottles plugged with cotton-wool at the outside temperature undergo little or no diminution in number for 48 hours, but at the end of a week there is a great diminution in number, which is still more marked at the end of 2 weeks. This was observed whether the water was a pure filtered water or contained a considerable amount of vegetable organic matter (*e.g.* a peaty water). The diminution in numbers appeared to be still more marked when the ordinary water organisms were also present.

The presence of *B. coli* in spring waters will next be considered (see Tables XIII and XIV). Not a large number of samples were examined, but they were derived from 8 distinct supplies. In only a single instance was *B. coli* isolated from the water in 50 c.c. In one other sample a positive neutral-red reaction was obtained, but no *B. coli* could be isolated though probably present. It is also to be noticed that in Table VII the two samples in which no *B. coli* were present were pure spring waters. Supply No. 20 possesses peculiar features and will be considered by itself.

It is of interest to compare with these results those from water supplies which are mainly spring water but into which a certain amount of upland surface water gains access. Examples of such waters are given in Tables XI and XII.

The results obtained are very similar to those of spring water alone, but as might be anticipated *B. coli* are slightly more frequently found.

The greater the amount of upland surface water the greater the probability of finding *B. coli* in the amounts examined.

B. coli in shallow wells.—This organism is present in almost all, and usually in considerable numbers. The majority were liable to pollution. No. 19 however is said not to be liable to pollution.

What is the significance to be ascribed to the presence of *B. coli* in these different classes of waters?

In entirely spring water my results agree with those of other workers and it seems a fair standard to expect that *B. coli* should not be discoverable in at least 50 c.c. of the water.

In shallow wells their presence must indicate either surface water and washings gaining access, or insufficient filtration through the soil. When the source of such water and its possibilities of contamination are considered it seems not unreasonable to regard their presence in anything like large numbers with much suspicion. The interpretation must be undertaken with a knowledge of the possibilities of contamination and other points of importance. I am averse to arbitrary standards, but the discovery of *B. coli* in as little as 10 c.c. would raise suspicion; and finding them in 2 c.c. would certainly lead me to pronounce strongly against the suitability of the water for drinking purposes.

The significance of *B. coli* in upland surface waters is a matter of considerable difficulty, but one of great interest. In my experience they are present in *all* upland surface supplies and usually in considerable numbers. This is quite apart from possibilities of contamination from human sources, and must be ascribed, as already explained, to contamination from animal excreta.

Can such pollution be considered harmful?

Sheep are allowed to graze on many of the best gathering areas in the country. Sheep and other animals do not suffer from enteric fever or other specific disease transferable to man by water, and it is difficult to see how their presence can be looked upon as harmful.

Professor Boyce remarks (8), "Although the *B. coli* is normally found in the intestine of man and animals, and therefore cannot be

said to be under these circumstances harmful, nevertheless cases do occur in which marked diarrhoea is found associated with great development of this organism in the intestine."

There is however no evidence as far as I am aware that *B. coli* as such and present in water has set up disease by its ingestion, while in Glamorganshire many millions of *B. coli* are daily consumed with no apparent harm.

Houston (6) states, "It cannot be denied that *B. coli* is present in the evacuations of many animals, but we have yet to learn that the excreta of animals are altogether harmless to man." Also Horrocks (12) remarks, "It is not justifiable to assume that the excreta of animals are harmless to man; and in any case a water so polluted could not be considered desirable for drinking purposes."

These remarks appear to me to beg the question at issue.

The value of the detection of *B. coli* in water is pre-eminently that it is *an indication* of contamination by sewage or other material which may contain the actual micro-organism of specific diseases—more particularly the *Bacillus typhosus*.

It is generally accepted that it is not the *B. coli* which are themselves harmful but that they merely serve as indicators of possible contamination with specific organisms.

In upland surface waters my figures point to the conclusion (as far as they go) that *B. coli* cannot be considered such an indicator for these waters, and so much of its value falls to the ground.

Its presence in upland surface waters even in large numbers (*i.e.* 500 per litre) may, and apparently not infrequently does, indicate contamination by animal excreta, a contamination possibly objectionable but causing and indicating danger in no way comparable to the danger caused by contamination with sewage.

I am of opinion that it is particularly unreliable to adopt any arbitrary standard for *B. coli* in upland surface waters. Each case must be considered on its merits.

To state, for example, that because of its proved presence in say 10 c.c. of a water that water should be condemned as showing dangerous contamination would in my opinion be a very unreliable and an unjustifiable deduction, and would, at least for Glamorganshire, condemn many of the best waters in the country.

It is to be noticed also that with the same water (*e.g.* the same reservoir) this organism has been present sometimes in 2, 10 and 40, while at others absent in these amounts or only present in 40 c.c.

while the supply itself has remained free from possibility of contamination other than from animals grazing.

Also the number of *B. coli* varies in different parts of the same reservoir (*vide supra*, for Reservoir C, inlet, middle, and outlet samples). Here arbitrary standards might condemn at one time and not at another, at one part of the reservoir and not at another.

The examination for this organism in upland waters has, I believe, its value, but less than is usually ascribed to it. When found in large numbers such as several per c.c. it may be justifiable to condemn the water as unsuitable for drinking purposes, but if in smaller numbers such a deduction is one not to be made lightly, and may easily be unjustifiable. Thus for supply No. 9 the tables show that *B. coli* was more numerous than in pure upland waters such as Nos. 1—3; but the numerical difference was not sufficiently marked to enable, from this factor alone, a deduction to be made that one water was good and the other bad.

There are some further points of importance which may be mentioned. Supply No. 20 shows features of interest. In supply No. 20 *B. coli* have almost invariably been found, often in very considerable numbers. This supply is said to be a deep well or spring, and there is no doubt that in the sense of the well passing through impermeable strata this is the case.

The source of the very large quantity of water available is somewhat of a mystery. The statement has been made, but I cannot be certain as to its accuracy, that since so much water has been pumped from this supply many of the surface wells in the neighbourhood have become dry. Two examinations made of the soil are of interest. Both samples were collected with proper precautions 6 inches beneath the surface. One sample was taken from near the end of the heading near the Penstock chamber, and therefore from soil liable to flooding from the contaminated brook. The other from soil of the same nature but not liable to flooding. The first sample (from near Penstock) showed about 3,280,000 organisms and *B. coli* was readily isolated (about 400 per gramme), while the control soil showed about 1,360,000 organisms and no *B. coli* were found (in 0.025 gm. examined¹).

¹ More recently (April 14, 1902) 2 fresh soil samples were examined for *B. coli*. In sample *A*, taken from over the heading 6 inches beneath the surface, *B. coli* was isolated from 0.005 gm. of soil, and in sample *B* some distance away it was found in about 0.25 gm. of soil but not in 0.005 gm.

Turning to the bacteriological examinations an obvious feature is the large number of organisms present, and particularly the extraordinary fluctuation in the number of organisms.

This is probably to be ascribed to the suction action of powerful pumps. A further point is that the Bismark-brown *Cladothrix* (Houston) is present not infrequently in this water, an organism rare in most waters.

There seems reasonable ground then for believing that this water, whatever its exact nature, is contaminated by surface water unpurified by filtration through sufficient soil, and by surface water from undesirable sources. I have repeatedly condemned this water on these grounds. The comparison of the chemical and bacteriological analysis for this water is particularly instructive. Chemically it is a water of extreme hardness, but organically it shows no evidence of contamination. The free ammonia is very small, while the albuminoid ammonia, though larger than usually met with in deep well waters, is not high. The chemical results show a very considerable uniformity, though the analyses recorded extend over $1\frac{1}{2}$ years. This is in marked contrast to the bacteriological results. It is however to be noted that if the 6 samples are compared in which chemical and bacteriological examinations of identical samples were made, there is an almost exact parallel between the number of organisms present and the organic purity as shown by the two ammonia figures.

In regard to chemical *versus* bacteriological examination it is not my purpose here to make any elaborate comparison. A large number of analyses are available the majority of which are taken under strictly comparable conditions, *i.e.* at the same time and from exactly the same place. In general it will be noticed that the chemical figures show much less fluctuation and variation from season to season, and the method appears to be much less sensitive.

A water which is contaminated sufficiently to yield evidence of such pollution by chemical analysis will usually show overwhelming evidence pointing to the same conclusion on bacteriological examination, while many waters on the other hand show pollution by bacteriological methods which on chemical analysis alone are above suspicion.

The bacteriological data require however much greater skill and experience to interpret, while the possibility of false deductions from faulty collection or local contamination is very much greater.

A criticism not infrequently applied to tabular results as given

above is that the organisms which are called *B. coli* are not all really that organism but include many organisms present often in good waters and not significant at all of contamination. Thus Horrocks (*loc. cit.* p. 104) says, "The statement that *B. coli* exists abundantly in all waters and soils appears to be based on a very elastic interpretation of the characteristics of *B. coli*."

To answer such a criticism the organisms isolated were all sufficiently worked out, and it is to be noticed that *B. coli* was isolated in all cases unless otherwise indicated, and that all negative neutral-red results were examined in exactly the same way as positive ones to be certain of the absence of the organism in question.

The characters of the *B. coli* isolated are indicated by the small letters in the column of the tables marked "Characters of *B. coli* isolated." They all had certain characters in common.

Under group *a* are included quite typical *B. coli*, *i.e.* organisms which give typical or at least possible surface colonies on agar or gelatine plates, which do not liquefy gelatine, but grow readily on a gelatine slope, generally as a bluish semitranslucent growth, which have a possible morphology (bacilli with rounded ends, and mostly quite short bacilli), are motile, usually very sluggish but occasionally more actively motile, which give uniform turbidity in peptone broth, and which give *all* the three following chemical tests, gas production in glucose media (agar-shake preparations used), milk coagulation with acid production, and the development of indol.

Under group *b* are included organisms quite similar to the above but which produce no gas in sugar media; under group *c* as above, but do not coagulate milk; and under group *d* also as group *a* except that no indol is produced.

Under group *e* are included the organisms which do not fall into any of the above groups and which are doubtful *B. coli*.

An examination of the Tables given shows that out of 95 *B. coli* isolated, 71 are included under group *a* (74.7 p.c.), 1 under group *b* (1.05 p.c.), 14 under group *c* (14.7 p.c.), 7 under group *d* (7.4 p.c.), and 2 under group *e* (2.1 p.c.).

Of these I think all bacteriologists would accept groups *a*, *b*, *c*, and *d* as *B. coli*. The group *e* are however doubtful, so their characters are given briefly.

Supply No. 1, No. 5. Short bacilli with rounded ends; not stained by Gram's method. No spores. Sluggishly motile. Uniform turbidity in peptone broth, on gelatine slope a white growth showing no lique-

faction. Grown in litmus milk, acid production but no coagulation (3 weeks), glucose neutral-red shake, no gas and no neutral-red reaction, Laetose agar-shake gives a small amount of gas, neutral-red broth no colour changes. Tested for indol in 10 days' old peptone broth it gave a slight red reaction.

Supply No. 6, No. 4. Short bacilli, not stained by Gram's method. No spores. Fair motility. Uniform turbidity in peptone broth. On gelatine slope gives a white translucent growth with no liquefaction. Grown in litmus milk, acid production but no coagulation (4 weeks). Glucose neutral-red shake, no gas and no neutral-red reaction but produces gas in lactose agar-shake preparations. No indol reaction was given with a 9-day broth culture but a moderate amount was demonstrated in a 10-day old peptone water culture.

Whether these two organisms would be accepted by bacteriologists as non-typical *B. coli* does not affect to any extent the points under consideration.

It will be noticed that 155 bacteriological examinations are recorded. Of these 16 were not examined for *B. coli*, and in 10 the neutral-red reactions are given, but the organism was either not looked for further or the method used failed to isolate it.

Of the 129 examinations left, in 34 *B. coli* was absent, in 93 certainly present, while in 2 a doubtful organism was isolated.

It may also be contended that the method used was a selective one and tended to pick out the *B. coli* which could reduce neutral-red and so does not give a fair measure of the distribution of this organism. To this it may be answered that almost all *B. coli* will reduce neutral-red to a greater or less degree and that in any case the negative results were also brushed and examined.

CONCLUSIONS.

1. In estimating the significance of *B. coli* in a sample of water the particular kind of water must be carefully considered, also the exact part of the system from which the sample is taken.

2. The number of *B. coli* present is an essential factor, but arbitrary standards of the number of this organism allowable per litre are of but little value and are fraught with considerable possibilities of error unless the particular kind of water and the local conditions are considered in every case.

3. Waters which show no *B. coli* in 50 c.c. are of a high degree

of purity, and therefore the proved absence of this organism in this amount, and still better in larger quantities, is of great value.

4. *B. coli* should be absent from at least 50 c.c. of spring water, possibly from greater amounts.

5. In upland surface waters the presence of *B. coli* in 40, 10 or even 2 or 1 c.c. means contamination, but not necessarily a contamination which it is essential to prevent. It may be from contamination with the excreta of animals grazing on the gathering areas and is by no means necessarily from sewage or other material containing specific organisms of infection. Further there is no evidence that an amount of such animal contamination sufficient to cause a considerable number of *B. coli* per litre to be present in the water is harmful.

If *B. coli* are present in numbers greater than say 500 per litre (or even in that amount) such a water is suspicious as it is rare to get so many *B. coli* in a water purely from the kind of animal contamination indicated, and further investigation is desirable. In filtered samples the number of *B. coli* is as a rule considerably reduced.

6. Chemical analysis cannot be considered a delicate method of detecting organic contamination, because it may fail with many waters in which pollution is undoubtedly taking place.

7. In surface wells *B. coli* in large numbers indicate surface or other contamination generally very undesirable if not actually dangerous. A knowledge of the position and the possibilities of contamination is very desirable in giving an opinion as to the purity of the water.

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- (12) *Ibid.* p. 104.

TABLE I.
Supply No. 1. Samples from gathering areas.

Date examined	No.	Source	No. of organisms per c.c. developing at		If <i>B. coli</i> present in ⁽³⁾					Characters of <i>B. coli</i> isolated	Remarks
			37° C.	20° C.	1/2	1	2	10	40 c.c.		
June 3, 1901	1	Reservoir A ⁽¹⁾	2	61					-	a ⁽⁴⁾	
July 8, "	2	"	1	24					-	a	
Sept. 16, "	3	"	11	160			-	-	+	e	4 c.c. also negative
Nov. 11, "	4	"	8	218			-	+	+	a	
Jan. 31, 1902	5	"	144	1270	-	-	+	+	+	a	
Feb. 27, "	6	"	42	850	-		+	+	+	a	
Mar. 29, "	7	"	75	1040	-		-	+	+	a	
Aug. 8, 1901	8	The main stream entering Reservoir A	14	201					+	a	
Sept. 16, "	9	"	616	1440		+		+	+	a	
June 3, "	10	Reservoir B ⁽¹⁾	16	136				- (6 c.c.)	+	a	
July 8, "	11	"	0	14					-	a	
Sept. 16, "	12	"	9	198			+	+	+	a	4 c.c. also gave positive result
Nov. 11, "	13	"	36	410			+	+	+	a	
Jan. 31, 1902	14	"	36	1105	-	+	-	+	+	c	
Feb. 27, "	15	"	65	995	-		+	+	+	a	
Nov. 11, 1901	16	The largest stream entering Reservoir B ⁽²⁾	132	350		-	-	-	+	a	Rapid liquefaction of gelatine plates
"	17	A small mountain stream entering Reservoir B	4	200			-	-	+	a	4 c.c. also negative

⁽¹⁾ All samples from these reservoirs were taken about 20 yards from the shore and from beneath the surface to as far as possible avoid local contamination.

⁽²⁾ Rises 2 miles away among the hills and no possibility of contamination apart from sheep.

⁽³⁾ + = *B. coli* present; - = *B. coli* absent.
⁽⁴⁾ The letters refer to the group of *B. coli*, vide text.

TABLE II.
Supply No. 1. Samples from storage reservoirs.

Date examined	No.	Source	No. of organisms per c.c. developing at		If <i>B. coli</i> present in					Characters of <i>B. coli</i> isolated	Remarks
			37° C.	20° C.	1/2	1	2	10	40 c.c.		
June 13, 1901	1	Reservoir C ⁽¹⁾	37	282					+	a	
July 29, "	2	" C	7	170				-	+	a	
Sept. 12, "	3	" C	5	170				-	-		(2)
Nov. 29, "	4	" C	5	118			-	+	+	a	
Jan. 29, 1902	5	" C	3	132			-	+	+	a	
Feb. 26, "	6	" C	3	94			-	-	+	a	
June 13, 1901	7	" D ⁽¹⁾	33	274				-	+	a	
July 29, "	8	" D	3	152				+	+	a	
Sept. 12, "	9	" D	48	268				+	+	a	
Jan. 29, 1902	10	" D	3	260			+	+	+	a	(2)
Feb. 26, "	11	" D	23	156	-		-	-	+		(2)

⁽¹⁾ Collected near to outlet from reservoir. This applies to all the samples in this table.

⁽²⁾ Positive neutral-red reaction but not examined further for *B. coli*.

B. coli in Drinking Water

TABLE III.

Supply No. 1. Samples from filter beds and service taps.

Date examined	No.	Source		No. of organisms per c.c. developing at		If <i>B. coli</i> present in				Characters of <i>B. coli</i> isolated	Remarks
				37° C.	20° C.	1/2	2	10	40 c.c.		
June 11, 1901	1	E filter beds.	Filtered water	0	22				-	a	
July 29, "	2	"	"	1	39			-	+		
Sept. 25, "	3	E filter beds.	Unfiltered water	2	331		-	-	-	(1)	<i>B. coli</i> also found in the sand of the filter beds
"	4	"	Filtered water	1	103			+	+	a	
Oct. 10, "	5	"	Unfiltered water	4	115			-	+	c	
"	6	"	Filtered "	9	80		-	-	+		
"	7	"	"	1	42			-	-		
Nov. 29, "	8	F filter beds.	Filtered water	2	38		+	+	+	a	
July 29, "	9	"	Unfiltered water	1	108		+	+	+	a	
Sept. 25, "	10	"	Filtered ⁽²⁾	31	6400		+	+	+	a	
Jan. 28, 1902	11	"	"	252	500		-	-	+	(1)	5 c.c. also +
Feb. 26, "	12	"	"	0	30		-	-	+	a	Rapid liquefaction of gelatine plates
Sept. 18, 1901	13	Filtered water from a service tap ⁽³⁾	"	1	34		-	-	+	c	
Oct. 1, "	14	"	"	9	290		-	-	-	a	<i>B. coli</i> isolated from 100 c.c.
Jan. 3, 1902	15	"	"	1	188				+	a	
July 2, 1901	16	Filtered water. Service tap in another town supplied	"	12	510		-	+	+	a	
Oct. 7, "		"	"								

⁽¹⁾ Positive neutral-red reaction but *B. coli* not isolated.⁽²⁾ Beds and tanks disturbed by making of fresh filter bed.⁽³⁾ Water allowed to run for 10 mins. before collection. Personally collected. About 2 Bismark-brown *Cladotrix* per c.c. also present.

TABLE IV.

Supply No. 1. *Chemical Analyses. (Summary of some analyses.)*

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Date examined	No.	Source	Corresponding bacteriological examination	Appearance in 2 ft. tube and reaction	In parts per 100,000						Sediment	Remarks
					Total hardness	Chlorine	Free ammonia	Albuminoid ammonia	Nitrites	Nitrates	Phosphates	
1900												
Dec. 17	1	Reservoir A		yellow, clear, alkaline	2.3	0.7	0.004	0.0102	Nil	Nil	Nil	Fair amount. Mainly vegetable debris
" 17	2	" B		" "	2.4	0.7	0.0022	0.0099	"	"	"	"
" 17	3	Small stream taking drainage of house near Reservoir A		yellow, turbid, alkaline	2.2	0.75	0.0036	0.0118	"	"	"	Considerable. Sand & vegetable debris
" 5	4	Reservoir C		yellow-green, alk.	4.1	0.75	0.0024	0.0108	"	"	"	Small amount. Vegetable tissue
" 5	5	" D		" "	4.3	0.8	0.0022	0.0128	"	"	"	"
" 6	6	E filter beds. Filtered water		" "	4.3	0.8	0.0018	0.0062	"	"	"	Practically nil
" 6	7	F " "		" "	3.7	0.75	0.0022	0.0096	"	"	"	"
1901												
April 3	8	Reservoir A		yellow, alkaline	2.5	1.0	0.0022	0.0124	"	"	"	Fair amt. Veg. debris, sand, animalculae, etc.
" 3	9	" B		" "	2.4	1.0	0.0024	0.0134	"	"	"	"
" 8	10	" C		" "	3.0	1.0	0.0036	0.0134	"	"	"	"
" 8	11	" D		" "	3.1	1.0	0.0024	0.0142	"	"	"	"
" 8	12	E filter beds. Filtered water		" "	3.3	1.0	0.0032	0.0104	"	"	"	Very little. Few animalculae
" 8	13	F " "		" "	3.6	1.1	0.0018	0.0076	"	"	"	"
1902												
Jan. 10	14	Reservoir A		yellow-green, alkaline, turbid	2.8	0.8	0.0031	0.0086	"	"	"	Very slight
" 10	15	" B		" "	3.2	0.9	0.0032	0.0082	"	"	"	"
" 9	16	" C		yellow, alkaline	3.7	0.9	0.0026	0.0076	"	"	"	Traces only
" 9	17	" D		" "	3.2	0.9	0.0032	0.008	"	"	"	Fair amt. Veg. debris
" 9	18	E filter beds. Filtered water		yellow-green, alk.	3.5	0.9	0.0024	0.0084	"	"	"	Practically nil
" 9	19	F " "		" "	3.2	0.9	0.0018	0.0084	"	"	"	"

TABLE V.
Supply No. 2. Bacteriological Examinations.

Date examined	No.	Source	No. of organisms per c. c. developing at		If <i>B. coli</i> present in				Characters of <i>B. coli</i> isolated	Remarks
			37° C.	20° C.	1/2	2	10	40 c. c.		
Jan. 25, 1901	1	Tap in one of the towns supplied	49	173						Not examined for <i>B. coli</i>
May 17, "	2	" " "	4	138					c	" "
July 16, "	3	" " "	192	370					a	
Oct. 9, "	4	Water just before filtration	41	218		+	+	+	a	Collected from covered tank which receives the filtered water
" "	5	Water filtered through quartz filters	48	120		+	+	+	d	Filtered through quartz filters
" "	6	Water filtered through sand filters	7	104		-	+	+	a	Taken near outlet
Oct. 30, "	7	Tap in one of the towns supplied	7	270						
Dec. 9, "	8	The main reservoir	204	1220		-	+	+	a	
" "	9	Marsh water	-	about 1500		+	+	+	a	
" "	10	Stream entering river	20	320		-	+	-	a	5 c. c. - also
" "	11	River between the two reservoirs	-	-		-	+	+	a	
" "	12	Stream entering river	27	144		+	+	+	a	
" "	13	" "	3	330		-	-	+	a	
" "	14	Just before quartz filtration	350	1250		-	+	+	a	

TABLE VI.
Supplies Nos. 2, 4 and 6. Chemical Analyses.

Date examined	No.	Source	Corresponding bacteriological examination	Appearance in 2 ft. tube and reaction	In parts per 100,000							Sediment	Remarks
					Total hardness	Chlorine	Free ammonia	Albuminoid ammonia	Nitrates	Nitrites	Phosphates		
1901		<i>Supply No. 2</i>											
July 30	1	Town service tap	—	yellow-green, alk.	2.6	1.0	0.0016	0.007	Nil	Nil	Very slight	Unsatisfactory degree of purity	
Oct. 9	2	Just before filtration	4	" "	4.1	0.9	0.0048	0.0092	"	"	Fair amt. Veg. debris & numerous animalculae		
"	3	After filtration thro' sand filters	6	" "	5.5	0.9	0.0024	0.005	"	"	Nil		
"	4	Town service tap	7	" "	8.9	1.1	0.0016	0.0042	"	"	Nil		
1901		<i>Supply No. 4</i>											
Jan. 12	1	Storage reservoir	1	yellow, neutral	3.2	1.6	0.0042	0.0056	Nil	Nil	Small. Veg. debris and numerous animalculae	Total solids = 7.3	
June 21	2	Service tap	2	" "	3.6	1.5	0.0512	0.0114	"	trace	Considerable amount. Mainly vegetable	Marked evidence of contamination. Possibly due to a local cause	
Nov. 14	3	"	4	yellow-green, alk.	4	1.6	0.0016	0.006	"	"	Nil		
1902													
Mar. 6	4	"	7	yellow-green, neutral	3.5	1.6	0.002	0.0062	trace	"	Nil		
1901		<i>Supply No. 6</i>											
Mar. 16	1	Service tap	2	yellow-green, neutral	3	1.05	0.0024	0.0044	Nil	Nil	Nil	Total solids = 4.8	
1902													
Jan. 21	2	"	4	yellow-green, alk.	3.7	1.0	0.0014	0.004	"	"	Nil		
Mar. 25	3	"	5	" "	4.7	1.0	0.0014	0.0044	"	"	Nil		

No Chemical Analyses available for Supplies 3 and 5.

TABLE VII.
Supply No. 3. Bacteriological Examinations.

Date examined	No.	Source	No. of organ- isms per c.c. developing at		If B. coli present in					Characters of B. coli isolated	Remarks
			37° C.	20° C.	1/10	1/2	2	10	40 c.c.		
Jan. 13, 1902	1	River on one side of the gauge	4	226	-	-	-	+	+	a	
"	2	River on the other side of gauge	3	218	-	-	-	-	+	a	
"	3	Spring running into river	2	65	-	-	+	-	+	c	
"	4	River; top of proposed reservoir	2	188	-	-	-	+	+	a	
"	5	Spring at upper part of area	4	458	-	-	-	-	-		
"	6	Another spring at upper part of area	2	30	-	-	-	-	-		

Note. Samples were collected about 18 hrs. before they were examined. They were not packed in ice. The weather was very cold throughout and the samples were kept outside all night (temperature below 0° C. all the time), so that though possibly some numerical multiplication took place it was probably slight, while the number of *B. coli* present, if altered at all, would presumably be reduced.

TABLE VIII.
Supplies Nos. 4, 5 and 6. Bacteriological Examinations.

Date examined	No.	Source	No. of organisms per c.c. developing at		If <i>E. coli</i> present in				Characters of <i>E. coli</i> isolated	Remarks	
			37° C.	20° C.	1/2	2	10	40 c.c.			
<i>Supply No. 4</i>											
Jan. 12, 1901	1	Storage reservoir	15	282						c	(1) Collected 250 yards above reservoir (2)
June 21, "	2	Service tap in the town	9	150				+	+		
July 12, "	3	" "	24	128				+	+	a	
Nov. 14, "	4	" "	27	270				+	+	a	
" 28, "	5	Reservoir	4	176				-	+	c	
" " "	6	Main inflowing stream to reservoir	5	124					+		
March 6, 1902	7	Service tap in the town	2	165				-	+		
<i>Supply No. 5</i>											
June 14, 1901	1	Service tap in the town (filtered)	2	102					-		Had been previously a considerable scarcity of water
Nov. 21, "	2	" "	11	352				+	+	a	
Feb. 18, "	3	" "	9	730			-	-	+	a	
<i>Supply No. 6</i>											
Feb. 11, 1901	1	Service tap (filtered)	10	71							a e a
March 16, "	2	" "	4	73						+	
July 2, "	3	" "	7	88						-	
Jan. 21, 1902	4	" "	3	80						-	
March 25, "	5	" "	0	40			-			-	

(1) Positive neutral-red reaction with the 10 and 40 c.c. but not examined for *B. coli*.
 (2) *B. coli* could not be found. Probably present but missed as no other neutral-red reacting organism found.

TABLE IX.

Supplies Nos. 7, 8 and 9. Bacteriological Examinations.

Date examined	No.	Source	No. of organisms per c.c. developing at		If <i>B. coli</i> present in					Characters of <i>B. coli</i> isolated	Remarks	
			37° C.	20° C.	1/10	1/2	2	10	40 c.c.			
<i>Supply No. 7</i>												
May 9, 1901	1	Service tap in the town	94	292								
" 27, "	2	" "	78	225						+	+	d
Oct. 9, "	3	" "	1	125						+	+	a
Jan. 21, 1902	4	" "	17	270					+	+	+	d
April 3, "	5	Storage reservoir	35	76	-	-	-	-	-	-	-	d
" "	6	Service tap in the town	7	94								
<i>Supply No. 8</i>												
Nov. 4, 1901	1	Tap	0	140								
March 27, 1902	2	From the service tank	9	234					-	-	-	a
<i>Supply No. 9</i>												
May 15, 1901	1	Filtered water from Reservoir C	11	172								
July 15, "	2	" " from Reservoirs A & B	9	114						+	+	d
Oct. 29, "	3	Taken from main river just before enters Reservoir C	3	64					-	-	-	c
March 3, 1902	4	enters Reservoir C and after two farm contaminated streams have entered it	plates overgrown	740		+			-	-	-	a
" "	5	Water overflow from Reservoir C	112	2800		+	+		+	+	+	a
" "	6	Reservoir C water just before filtration	plates overgrown	800		+	+		+	+	+	a
" "	7	Reservoir C water just after filtration	7	120		-			+		+	a
March 18, "	8	Water from main river above all but one of the farm contaminated streams	32	460		-			+		+	c

TABLE X.

Supplies Nos. 7, 8 and 9. Chemical Analyses.

Date examined	No.	Source	Corresponding bacteriological examination	Appearance in 2 ft. tube and reaction	In parts per 100,000							Sediment	Remarks
					Total hardness	Chlorine	Free ammonia	Albuminoid ammonia	Nitrites	Nitrates	Phosphates		
Supply No. 7													
1901	1	Service tap in the town	1	yellow, neutral	2.3	1.65	0.002	0.0132	Nil	Nil	Nil	Fair amount. Chiefly vegetable matter	
	2	" "	3	" "	3.4	1.6	0.006	0.0148	"	"	"	Small amount	
1902	3	" "	4	" "	3.3	1.6	0.0012	0.008	"	"	"	Practically nil	
	4	Storage reservoir	5	" "	3.3	1.65	0.005	0.0158	"	"	"	Fair amt. Veg. cells & debris. Numerous animalculae	
	5	Service tap in the town	6	yellow-green, neut.	3.3	1.7	0.0016	0.0082	"	"	"	Small amount	
Supply No. 8													
1901	1	Service tank		yellow-green, faintly alkaline	3.0	1.5	0.0052	0.0076	faint trace	Nil	Nil	Fair amount. Mainly vegetable debris	
	2	Tap	1	" "	2.7	1.6	0.0012	0.0044	Nil	"	"	Traces only	
1902	3	From service tank	2	yellow-green, neut.	3	1.7	0.004	0.0132	faint traces	"	faint traces	Fair amount. Veg. debris & numerous animalculae	
Supply No. 9													
1901	1	Filtered Reser. C water	1	yellow-green, alk.	5.3	1.0	0.0024	0.0062	Nil	Nil	Nil	Nil	
	2	" "	2	yellow, neutral	3.4	1.0	0.0034	0.0174	"	"	"	Fair amount. Veg. cells & debris	
	3	" " A & B "	3	" "	4	0.9	0.0014	0.0146	"	"	"	Small amount	
1902													
Mar. 3	4	Same as No. 4 bact. sample	4	yellow-green, alk.	4	1.0	0.002	0.0084	traces	"	"	Fair amount	
	5	Reservoir C overflow	5	" "	4	1.0	0.0036	0.0108	"	"	"	Considerable amt. Animalculae extremely numerous	
	6	Reservoir C just after filtration	7	" "	4	1.0	0.0044	0.0098	"	"	"	Small amount only	

TABLE XI.
Supplies Nos. 10, 11, 12, 13 and 14. Bacteriological Examinations.

Date examined	No.	Source	No. of organisms per c.c. developing at		If <i>B. coli</i> present in				Characters of <i>B. coli</i> isolated	Remarks	
			37° C.	20° C.	1/2	2	10	40 c.c.			
<i>Supply No. 10</i>											
Jan. 25, 1901	1	Tap in town supplied	1	45							
May 10, "	2	" "	1	126				-			
Nov. 1, "	3	" "	0	18				-			
Jan. 31, 1902	4	" "	3	65				-			
<i>Supply No. 11</i>											
Feb. 20, 1901	1	Supply tap	2	62							
Sept. 4, "	2	" "	3	14				-			
Jan. 6, 1902	3	" "	0	20				-			
Mar. 17, "	4	" "	1	82				+			<i>B. coli</i> present in Winchester quart of the water ⁽¹⁾
<i>Supply No. 12</i>											
Feb. 20, 1901	1	Tap in town supplied	1	46							
Nov. 20, "	2	" "	0	70				-		a	
Feb. 18, 1902	3	A tap in another part of the district	7	1190	-			-		c	
<i>Supply No. 13</i>											
Feb. 5, 1902	1	Tap	1	85				-			
<i>Supply No. 14</i>											
Feb. 6, 1902	1	Tap in town supplied	1	27				-			

⁽¹⁾ A positive neutral-red reaction with the 2 c.c. but not examined further. Probably an accidental contamination.

TABLE XII.

Supplies Nos. 10, 11, 12, 13 and 14. *Chemical Analyses.*

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Date examined	No.	Source	Corresponding bacteriological examination	Appearance in 2 ft. tube and reaction	In parts per 100,000							Sediment	Remarks
					Total hardness	Chlorine	Free ammonia	Albuminoid ammonia	Nitrates	Nitrites	Phosphates		
Supply No. 10													
1901 Jan. 25	1	Tap in town	1	almost colourless, neutral	3.5	1.0	0.0014	0.003	Nil	Nil	Nil	Nil	Total solids=5.2 Very high degree of organic purity Total solids=4.9
May 10	2	"	2	faint yellow-green, faintly alk.	3.4	1.2	0.001	0.0046	"	"	"	Small amount	
Nov. 1	3	"	3	yellow-green, neut.	3.0	1.3	0.0014	0.0042	"	"	"	Nil	
1902 Jan. 31	4	"	4	" alk.	3.8	1.3	0.001	0.0024	traces	"	"	Traces only	
Supply No. 11													
1901 Feb. 20	1	Service tap in town	1	yellow-green, neut.	2	1.0	0.0018	0.0038	traces	Nil	Nil	Slight	Total solids=8.8 All the samples show a high degree of organic purity
Sept. 4	2	" "	2	" "	3.3	1.05	0.0014	0.0038	Nil	"	"	Practically nil	
1902 Jan. 6	3	" "	3	" "	2.5	1.0	0.0014	0.003	"	"	"	"	
Mar. 17	4	" "	4	" "	3	1.05	0.001	0.003	traces	"	"	"	
Supply No. 12													
1901 Feb. 20	1	Tap in town supplied	1	yellow-green, alk.	3.2	1.1	0.002	0.0042	Nil	Nil	Nil	Practically nil	
Nov. 20	2	" "	2	" "	6	1.1	0.001	0.0026	"	"	"	A small amount	
1902 Feb. 18	3	Tap in different district	3	" "	6.7	1.0	0.001	0.0032	traces	"	"	Considerable amt. Veg. debris and a few animalculae	
Supply No. 13													
1900 Dec. 14	1	Tap	—	yellow-green, neut.	3.9	2.2	0.0016	0.0022	Nil	Nil	Nil	Small amount	A high degree of organic purity
Supply No. 14													
1902 Feb. 6	1	Tap in town supplied	1	yellow-green, neut.	4	1.3	0.0016	0.0024	faint traces	Nil	Nil	Nil	" " "

TABLE XIV.

Supplies Nos. 15, 16 and 17. Chemical Analyses.

Date examined	No.	Source	Corresponding bacteriological examination	Appearance in 2 ft. tube and reaction	In parts per 100,000						Sediment	Remarks
					Total hard- ness	Chlo- rine	Free ammonia	Albu- menoid ammonia	Ni- trates	Ni- trites	Phos- phates	
1902 Mar. 20	1	<i>Supply No. 15</i> The covered reservoir	4	yellow-green, alk.	14.8	1.1	0.004	0.0102	traces	Nil	Nil	Traces only
" "	2		5	" "	15.4	1.1	0.0014	0.0036	"	"	"	
1901 July 8	1	<i>Supply No. 16</i> Tap in town supplied	1	yellow-green, alk.	7	1.45	0.0018	0.0026	traces	Nil	Nil	Practically nil
1902 Feb. 6	2		2	" "	8.8	1.4	0.0010	0.0020	"	"	"	
1901 Oct. 28	1	<i>Isolated Samples from different sources (No. 17)</i>			13	2.9	0.0012	0.0042	traces	Nil	Nil	High degree of organic purity
1902 Feb. 18	2				4.6	1.5	0.0014	0.0024	"	"	"	

Very high albuminoid ammonia for this class of water and this is unsatisfactory. This is not shown in the tap specimen
High degree of organic purity
" " "
" " "

High degree of organic purity
" " "

TABLE XV.
Shallow Wells. Bacteriological Examinations.

Date examined	No.	Source	No. of organisms per c.c. developing at		If <i>B. coli</i> present in				Characters of <i>B. coli</i> isolated	Remarks	
			37° C.	20° C.	1/2	2	10	40 c.c.			
<i>Isolated Samples from different sources (No. 18)</i>											
June 26, 1901	1	A shallow well	6	107			-	-	a	Gelatine plates rapidly liquefied and fig. represents only averages of the 2 day counts. (1) Same water as No. 2	
July 1, "	2	Pump water. Shallow well	202	1120			+	+			
" 2, "	3	A shallow well	1080	about 10,000			+	+	a		
" 7, "	4	" "	4	254			+	+	a		
" 22, "	5	" "	140	980			+	+	a		
Oct. 22, "	6	A well water	117	2100			+	+	a		
" 30, "	7	A surface well	19	480		+	+	+	d		
" 15, "	8	" "	1	56			-	+	a		
Nov. 4, 1902	9	" "	—	330		-(1 c.c.)	+	+	c		
" 23, "	10	" "	82	220		-(1 c.c.)	+	+	a		
Feb. 8, "	11	A well water	1370	1800	-	+	+	+	a		
" 5, "	12	A surface well	38	2400		+	+	+	b		
" 12, "	13	" "	130	1920	-	-	+	+			
Sept. 28, 1901	14	Pump water. Shallow well	25	104		+	+	+	a		
<i>No. 19. A shallow well</i>											
Jan. 22, 1901	1	From the pump	11	174			+	+	a	Gelatine rapidly liquefied and 20° C. count = average for 2 days	
Dec. 18, "	2	" "	40	580		+	+				
Mar. 17, 1902	3	" "	520	3100	+	+			c		

(1) A positive neutral-red reaction with the 10 c.c. but not examined further.

TABLE XVI.

Shallow Wells. Chemical Analyses.

Date examined	No.	Source	Corresponding bacteriological examination	Appearance in 2 ft. tube and reaction	In parts per 100,000						Sediment	Remarks
					Total hardness	Chlorine	Free ammonia	Albuminoid ammonia	Nitrites	Phosphates		
<i>Isolated Samples from different sources (No. 18)</i>												
1901 June 26	1	A surface well	1	yellow-green, alk.	10.2	1.85	0.0016	0.0066	traces	Nil	Small amt. Veg. deb. Animalculae fairly numerous	
July 1	2	"	2	"	29	2.5	0.0020	0.0048	marked traces	"	Fair amt. Chiefly veg. deb. Animalculae fairly numerous	
1902 Jan. 4	3	"	9	neut.	11.2	3.7	0.0032	0.0048	traces	"	Fair amt. Veg. deb. mainly	
" 23	4	"	10	alk.	26.4	2	0.0012	0.0028	marked traces	"	Practically nil	
Feb. 8	5	A well water	11	"	7	2.6	0.0058	0.0076	traces	Nil	Considerable amt. Mainly veg. deb. A few animalculae	
1901 Sept. 28	6	A surface well	14	"	28	2.5	0.0018	0.0048	distinct traces	"	Fair amt. Veg. deb. and a few animalculae	Same water as No. 2
<i>Supply No. 19</i>												
1901 Jan. 22	1	From the pump	1	yellow-green, alk.	32	2.1	0.0018	0.0052	traces	Nil	Traces only	Total solids=37.8 Volatile " = 6.9
Dec. 18	2	"	2	"	44	6.3	0.0024	0.0108	well marked	"	Small amount. Veg. deb. mainly	Permanent hardness 11.2
1902 Mar. 17	3	"	3	yellow, alkaline	43	5.6	0.0026	0.0130	1.2	traces	Small amount. Veg. deb. mainly	Evidence of contamination

TABLE XVII.
Supply No. 20. Bacteriological Examinations.

Date examined	No.	Source	No. of organisms per c.c. developing at		If <i>B. coli</i> present in					Characters of <i>B. coli</i> isolated	Remarks
			37° C.	20° C.	1/10	1/2	2	10	40 c.c.		
Oct. 13, 1900	1	Tap at pumping station	72	196							
Jan. 16, 1901	2	" "	136	408							
Feb. 14, "	3	" "	266	473				+ (5 c.c.)		a	5 c.c. only examined and from this <i>B. coli</i> isolated.
Mar. 22, "	4	" "	1980	2108						a	Fatal to a guinea-pig
" "	5	Tap in town supplied	484	1278							Not brushed or further examined for <i>B. coli</i>
May 16, "	6	Tap at pumping station	692	1780			+				(1)
June 25, "	7	Water in penstock valve chamber	7	42			-				
" "	8	Tap at pumping station	210	370			+	(partial)			
July 6, "	9	Water in penstock valve chamber	191	620				+ (5 c.c.)		a	Collected myself and started within an hour of being collected
" "	10	Tap at pumping station	135	402				+ (5 c.c.)		a	
Oct. 7, "	11	" "	5	60		+ (1 c.c.)	+			a	
Jan. 14, 1902	12	" "	30	130	-	-	+			c	
" "	13	Tap in town supplied	65	392	-	-	-			a	
" "	14	An accessory spring water flowing into one of the reservoirs	14	94			-			d	
Jan. 29, "	15	Another accessory spring in district	115	1420		+	+			a	
April 2, "	16	Tap at pumping station	150	780	-		+			a	

(1) A positive neutral-red reaction but not examined further.

TABLE XVIII.

Supply No. 20. Chemical Analyses.

Date examined	No.	Source	Corresponding bacteriological examination	Appearance in 2 ft. tube and reaction	In parts per 100,000						Sediment	Remarks
					Total hardness	Chlorine	Free ammonia	Albuminoid ammonia	Nitrites	Nitrates		
1900 Oct. 13	1	Tap at pumping station	1	yellow-green, alk.	37	2.6	0.0020	0.0044	0.47	Nil	Practically nil	Total solids=49.0
1901 Jan. 16	2	" "	2	"	36	2.5	0.0022	0.005	distinct traces	"	Small amount. A few animalculae	Total solids=38
Mar. 22	3	" "	4	"	38	2.5	0.0018	0.0088	"	"	Practically nil	
July 20	4	" "	"	"	38	2.9	0.0032	0.0078	"	"	"	
Oct. 7	5	" "	11	"	39	2.7	0.0018	0.0044	"	"	"	
1902 Jan. 14	6	" "	12	"	38.6	2.7	0.0016	0.0042	"	"	"	
" 7	7	Accessory spring	14	"	35	2.3	0.002	0.0078	Nil	"	Small amt. Veg. deb.	Total solids=38
Jan. 29	8	Another accessory spring	15	"	38.4	1.4	0.0014	0.0064	traces	"	Small amt. Veg. deb. and a few animalculae	
April 2	9	Tap at pumping station	16	"	38.4	2.6	0.0014	0.0048	distinct traces	"	Traces only	

